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Investigation into the microbial flora of healing and non-healing decubitus ulcers

DIANA C DALTREY, B RHODES, JG CHATTWOOD

From the School of Health and Applied Sciences, Leeds Polytechnic, Leeds LS1 3HE

SUMMARY Seventy-four pressure lesions in fifty-three geriatric patients were observed at weekly intervals to determine the bacterial flora and the healing index of each lesion, expressed as $\left[\frac{\text{initial area of lesion (cm}^2\text{)} - \text{final area of lesion (cm}^2\text{)}}{\text{time in days}}\right]$. The micro-organisms which caused infection included *Staphylococcus aureus*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Bacteroides fragilis* and *Bacteroides asaccharolyticus*. Many lesions contained a mixed flora. *P mirabilis* and *Ps aeruginosa* were associated with necrotic ($p < 0{\cdot}005$) and enlarging ($p < 5 \times 10^{-7}$) lesions. *Bacteroides* spp were associated with necrotic lesions ($p < 0{\cdot}05$). The presence of *S aureus* in a lesion was not associated with any particular trend in healing index. The implications of the microbiological findings are discussed.

Decubitus ulcers are known to harbour a flora of aerobic and sometimes anaerobic bacteria. However, there seems to be no consensus of opinion on how this may affect the healing process. Bendy *et al.*, in a study of topical gentamicin treatment, concluded that "suppression of growth of bacterial pathogens is the decisive factor in the healing of decubiti."[1] Morgan cites absence of pressure, debridement of dead tissue and control of infection as the three factors determining success of topical treatments.[2] The opposite view is summarised in a *British Medical Journal* leader "There is little place for antibiotics. Pressure sores, like varicose ulcers heal when the underlying cause is eliminated. Bacteria merely colonise the ulcer—they do not cause it."[3]

The aim of this investigation was to study the bacterial flora of a series of decubitus ulcers of varying severity and to try to relate the types and numbers of organisms present to the types and healing rates of the lesions.

Patients and methods

SUBJECT SAMPLE

Fifty-three patients (11 men, 42 women) admitted to the geriatric unit of a large general hospital were included in the study, which was carried out in conjunction with a clinical trial of karaya gum power (sterculia or Indian tragacanth).[4] Ages ranged from 64 to 97 yr (mean 79 yr). Decubiti were studied from the time the patient was admitted or the lesion identified until the lesion healed or the patient was discharged or died. (Range 1-47 wk, mean 5 wk.) Clinical data for the patients were collated.

Pressure lesions were classified as superficial, ulcerative, or necrotic by the nurse researcher. Most superficial and some ulcerative lesions were treated with Tincture Benzoin Co, Karaya, or Karaya with povidone iodine. Most necrotic and the more severe ulcerative lesions were treated with Eusol. Other treatments occasionally used were Paranet, Cicatrin, dextranomer, Aserbine and zinc sulphate.

In fourteen patients with several lesions, all were fully studied but only the largest lesion from each patient was included in the statistical analysis. Five patients were excluded from some of the analysis because no healing index could be calculated for reasons of medical management.

MEASUREMENT OF HEALING RATE

Lesions were measured initially, at weekly intervals, and when the patient left the study. Except on rare occasions, the nurse researcher made all the measurements. A transparent rule was used to measure the longest wound axis in millimetres (A) and a second measurement was made at right angles to the first (B). Both measurements passed through the midpoint. The area of the lesion was taken to be $\pi\frac{1}{2}A\frac{1}{2}B$. This method of measurement was adopted since a planimeter was not available for most of the study. To

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assess the error, twelve lesions were traced and measured by planimetry in parallel with the original method. Agreement between the methods was determined by linear regression analysis and was good ($r = 0{\cdot}92, p < 0{\cdot}001$).

Eventually a Healing Index (HI) was calculated for each lesion by subtracting the final area of the lesion from its initial area and dividing by the intervening time in days.

BACTERIOLOGICAL METHODS

Sampling

The semiquantitative sampling technique was designed to reflect comparative levels of colonisation in different lesions and in one lesion from week to week.

A serum-coated swab was moistened in sterile one quarter strength Ringer solution and used to swab an area of 1 cm^2 at the centre of the ulcer through a sterile metal template. The swab was aseptically broken off into 2 ml Ringer solution in a bijou bottle and the organisms eluted by vigorous shaking for exactly one minute. Serial tenfold dilutions of the eluate were made, and the count estimated by the method of Miles and Misra.[5]

Preliminary experiments showed that a count of $\geqslant 10^4/cm^2$ obtained by this method correlated with heavy growth on at least the first two quadrants of a streak plate prepared from a routine swab of the same lesion. Since it is virtually impossible clinically to assess damage due to infection in ischaemic lesions, the term infection is used to mean the persistent presence of a micro-organism at levels $> 10^4/cm^2$.

In twenty patients the anaerobic flora of the lesions was systematically investigated. A separate swab was taken from areas of the lesion judged most likely to yield anaerobes—for example, necrotic tissue, undercut edges, and this was immersed in Carey Blair anaerobic transport medium for transit to the laboratory. Conventional streak plates were prepared from these swabs and the plates assessed for significant levels of growth in the normal manner.

Isolation and identification

Routine counts were carried out on blood agar (Columbia agar base + 5% horse blood) MacConkey's agar and cetrimide agar (nutrient agar + 0·03% cetrimide). Aerobic plates were incubated at 37°C for 24 h before examination. Plates were then reincubated and checked after a further 24 h.

For anaerobic work the media used were vitamin K_1-blood agar (blood agar + 10 μg/ml vitamin K_1), neomycin-vitamin K_1-blood agar (blood agar + 10 μg/ml vitamin K_1 + 100 μg/ml neomycin base activity) and kanamycin-vancomycin-laked blood agar (Columbia agar base + 5% laked horse blood + 0·5 μg/ml kanamycin + 0·5 μg/ml vancomycin). These plates were either freshly poured or stored anaerobically and were warmed before inoculation. Anaerobic plates were incubated in 90% H_2, 10% CO_2 in Gaspak jars at 37°C for 48 h before examination. Plates were then reincubated anaerobically and checked a week after inoculation.

Aerobic bacteria were identified according to the methods of Cowan and Steel.[6] Anaerobic bacteria were identified according to the methods in the Wadsworth manual.[7] Staphylococci were phage-typed by the Public Health Laboratory Service. Streptococci were grouped using Phadebact reagents (ABCG) with Lancefield grouping by the acid extraction method for group D. Some group B streptococci were phage-typed by the Streptococcal Reference Laboratory, Colindale. *Ps aeruginosa* isolates were pyocine-typed by the method of Govan and Gillies.[8]

Results

Table 1 shows the range of organisms isolated. Normal skin flora such as *Staphylococcus epidermidis*, *Micrococcus* and coryneforms were not studied further. The aerobic and anaerobic bacteria which

Table 1 *Range of organisms isolated. Number of patients examined for aerobes: 53; for anaerobes: 20*

Aerobic bacteria	*No of patients*
Gram-positive cocci	
Staphylococcus aureus, S epidermidis	44, 31
Micrococcus spp	15
Streptococcus spp	31
A, B, C, D, G, other	1, 6, 6, 28, 6, 2
Gram-positive bacilli	
Coryneforms	31
Bacillus laterosporus	1
Gram-negative bacilli	
Proteus spp	27
mirabilis, morgani, rettgeri, other	25, 2, 1, 1
Escherichia coli	19
Klebsiella pneumoniae (sensu lato)	8
Citrobacter freundii	1
Hafnia alvei	1
Acinetobacter spp	2
Moraxella spp	2
Alcaligenes sp	1
Pseudomonas aeruginosa	15
Anaerobic bacteria	
Bacteroides fragilis group	7
fragilis, ovatus, thetaiotamicron, vulgatus, other	5, 1, 2, 1, 3
B asaccharolyticus	4
Fusobacterium necrophorum	1
Clostridium spp	5
perfringens, sporogenes, cadaveris	3, 1, 1
Gram-positive non-sporing rods	2
Gram-positive cocci	9
Yeasts	
Candida spp	3

Table 2 *Recovery of significant numbers of aerobes and anaerobes in relation to healing index and lesion type*

Micro-organism	*% lesions yielding micro-organism*				
	**Healing index*		*Lesion type*		
	⩽ 0	> 0	*Necrotic*	*Ulcerative*	*Superficial*
Aerobes	n = 14	n = 34	n = 12	n = 17	n = 24
Staphylococcus aureus	21	50	33	41	46
Streptococcus spp	14	12	25	6	17
Escherichia/Klebsiella spp	21	0	17	0	4
Proteus spp	57	3	50	12	8
Pseudomonas aeruginosa	43	6	50	18	4
Any aerobe	86	53	83	65	50
Anaerobes	n = 7	n = 10	n = 6	n = 5	n = 9
Bacteroides spp	57	10	67	40	0
Fusobacterium spp	14	0	17	0	0
Clostridium spp	14	10	17	20	0
Anaerobic cocci	0	10	33	0	0
Any anaerobe	57	10	67	40	0
Any micro-organism	86	53	83	65	50

*⩽ 0 static or enlarging lesions.
> 0 healing lesions.

caused infection are shown in Table 2. Gram-negative aerobic rods infected 71% of decubiti with HI ⩽ 0 compared to only 9% of resolving lesions. *Pseudomonas aeruginosa* and *Proteus mirabilis* were found most frequently. There was a significant association between *P mirabilis* and necrotic lesions ($p < 0.005$ χ^2 test) and *Ps aeruginosa* and necrotic lesions ($p < 0.005$ χ^2 test). Decubiti infected by either of these organisms had a significantly lower HI than decubiti not so infected (*P mirabilis* $p < 0.00003$, *Ps aeruginosa* $p < 0.006$; Mann Whitney *U* test). There was a highly significant association between *P mirabilis* or *Ps aeruginosa* or both and enlarging lesions (HI < 0) ($p < 5 \times 10^{-7}$ χ^2 test).

Twenty patients were examined for anaerobic flora. Six were infected with a mixture of aerobes and anaerobes and eight with aerobes alone. No lesion was infected by anaerobes alone. There was a significant association between anaerobic infection and necrotic lesions ($p < 0.05$, Fisher test). Decubiti infected by mixtures including anaerobic bacteria had a significantly lower HI than other lesions ($p < 0.025$ Mann Whitney *U* test). However *P mirabilis* and *Ps aeruginosa* were also present in some cases making interpretation difficult.

Comparison of lesions infected with *S aureus* alone with uninfected lesions showed no significant difference in HI between the groups. Fourteen different phage types and a number of non-typable isolates of *S aureus* were found; none was associated with any one category of lesion or with lowered HI. Many lesions were colonised transitorily rather than infected. Streptococci were also frequently present as transients. Nine patients only were infected with streptococci (six group D, one group C, one untyped and one group B). Indistinguishable strains of group B *Streptococcus* were isolated from the pressure lesion and the vagina of the last mentioned patient.

Altogether 20 lesions were uninfected, 15 were infected by a single organism (*S aureus* 12; *Ps aeruginosa* 2; *P mirabilis* 1) and 18 were infected by a mixture of organisms.

Povidone iodine was applied to superficial or ulcerative lesions in seven patients. There was no significant difference in either the incidence of infection or in the healing rate in patients treated with karaya + povidone iodine compared to those treated with karaya alone (Fisher test, Mann Whitney *U* test).

Since all patients with enlarging lesions died, clinical data were examined to establish whether these patients differed significantly in underlying illness, nutritional status, or age, from those with healing lesions.

The same range of illnesses occurred in both the patients who died and those who survived with the exception of carcinoma which occurred only in the former group. The most frequent diagnoses were cerebrovascular accident and bronchopneumonia: there was no significant difference in the incidence of these conditions between dying and surviving groups (χ^2 test). The incidences of diabetes, carcinoma, and anaemia are shown in Table 3. Weight loss occurred significantly more often in the group who died (χ^2 $p < 0.05$). Only two patients were on steroid treatment during the study and in both cases their lesions healed completely.

Patients who died were subgrouped into those with enlarging lesions and those with static or healing lesions: these groups were similar concerning underlying illness.

Discussion

Since numerous factors affect the healing process it is difficult to determine what effects, if any, are due to micro-organisms: the results should therefore be interpreted with caution.

The organisms particularly associated with necrotic lesions were *P mirabilis*, *Ps aeruginosa* and *Bacteroides* spp. Decubitus ulcers are ischaemic lesions and local resistance to infection will diminish as the lesion becomes more severe. Lack of local resistance and the presence of dead tissue may allow these organisms to gain a foothold. Anaerobes always occurred in combination with aerobes which may have helped to provide a suitable environment for

Table 3 *Clinical data in relation to patient survival and healing index of lesion*

	State of lesion in patients who died			*State of lesion in patients who survived*		
	Enlarging (HI < 0)	*Static (HI = 0)*	*Healing (HI > 0)*	*Enlarging (HI < 0)*	*Static (HI = 0)*	*Healing (HI > 0)*
No of patients	11	3	6	0	0	28
Mean age (yr)	78·3	79	78·8			79·4
No of patients showing:						
Weight loss	7*	0	2*			2
Anaemia (Hb < 10 g/dl)	1	1	2			5
Diabetes	1	1	0			2
Carcinoma	2	2	0			0
Healing index:						
Mean	− 211	0	13·8			15·3
Range	− 1024 to − 0·6	0	1·7 to 33·5			1·4 to 78·5
Class of lesion:						
Necrotic	6	0	2			2
Ulcerative	3	2	3			8
Superficial	2	1	1			18

HI = healing index.
*One patient on diuretics.

anaerobic growth in the decubitus. The range of anaerobes and their distribution in the lesion types are largely in agreement with the finding of Peromet.[9]

Properties of the micro-organisms themselves seem to be important, for Enterobacteriaceae other than *Proteus* spp only rarely became established in the mixed culture in enlarging lesions and never became established in any other lesions. Similarly, all members of the *Bacteroides fragilis* group were isolated but only *B fragilis* (formerly *B fragilis ss fragilis*) became established in the lesions.

The presence of *Proteus* spp and *Ps aeruginosa* cannot be entirely explained by a predilection for necrotic tissue. Only 6/11 enlarging lesions were classed as necrotic (see Table 3). Of the remaining five lesions, four contained *Proteus* spp/*Ps aeruginosa* and it was the least severe lesion in the group (HI −0·6) which was free of these organisms. Amongst patients with healing lesions *Proteus* spp or *Ps aeruginosa* infection or both was found in only 3/33 non-necrotic lesions.

Examination of clinical data did not reveal an obvious explanation for the enlarging lesions. All the patients in the study were old, but ages were similar in patients with enlarging and with healing lesions, as were the underlying illnesses. Of the four patients with carcinoma two had enlarging lesions infected by Gram-negative aerobes; the other two had static, uninfected lesions. Four patients had mild diabetes: one suffered a necrotic enlarging lesion infected by *Ps aeruginosa* and one an ulcerative lesion infected with *Bacteroides* spp and *Streptococcus* group D, which remained static. The other two diabetics had only superficial, healing lesions, one uninfected, the other infected with *S aureus*.

Of the twenty patients who died, nine had lost weight. Seven of these were in the group with enlarging lesions, suggesting an association of this condition with weight loss, but this did not reach significance at the 95% level (Fisher test). If weight loss is taken as an indication of poor nutritional status then this is not an invariable concomitant of an enlarging lesion, since the two patients with the most severe lesions of all were not losing weight and had a dietary intake classed as normal.

Although poor nutritional status would predispose patients to infection, an infection once initiated might contribute to general debilitation of the patient, and *Proteus* spp or *Ps aeruginosa* once established in a lesion might contribute to local damage thereby delaying or preventing healing.

Axler *et al.* found that Gram-negative rods present as predominant members of the flora of cutaneous ulcers, precluded porcine skin grafts from promoting healing. Predominantly Gram-positive flora on the ulcer surface allowed healing to take place, although *S aureus* retarded the process.[10]

We have no evidence to suggest that *S aureus* delays healing, but we were comparing lesions persistently colonised by $> 10^4$ *S aureus*/cm^2 with lesions containing less than this number, whereas Axler was looking for presence or absence of the organism.

The presence of large numbers of micro-organisms in decubiti is hazardous since there is a risk to the patient of invasion and septicaemia[11] and a risk to other patients of cross-infection.[12 13]

Pyocine typing of *Ps aeruginosa* and phage-typing of *S aureus* and *Streptococcus* group B suggested that some sharing of organisms occurred in this series of geriatric patients. Neglect of decubitus ulcers as a potential source of pathogens may lead to avoidable cross-infection.

We suggest that *Proteus* spp and *Ps aeruginosa*

infection of decubiti in elderly patients may be of greater clinical importance than presently acknowledged, and that this merits further investigation. It seems unwise to view such infections as "merely colonisation."

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Requests for reprints to: Dr Diana C Daltrey, School of Health and Applied Sciences, Leeds Polytechnic, Calverley Street, Leeds LS1 3HE, England.